respectfully traverse these rejections and address these rejections as they relate to the amended claim set filed herewith.

35 U.S.C.§112, second paragraph, indefiniteness:

In the Final Office Action dated January 3, 2002, claims 14-16 were rejected under 35 U.S.C. §112, second paragraph as allegedly indefinite. In particular, the Examiner states that it is not clear from the claims or the text of the specification how one of skill in the art would carry out a mixing step in a flow through mixer, a centrifugation step and a neutralization step simultaneously. The Examiner states that each step would appear to be mutually exclusive and would require a separate piece of equipment which would be difficult to combine for the simultaneous performance of all steps. Applicants respectfully disagree.

Although Applicants respectfully disagree with the Examiner's position for the reasons stated in the response filed October 9, 2001, Applicants amend the specification herein to add a new claim 22, dependent on claim 1 and reciting that the steps of claim 1 are performed in a continuous process on a single large sample. Claims 14-16 are amended to depend from new claim 22. Applicants submit that the claims as amended are definite.

35 U.S.C. § 103(a), obviousness:

In the Final Office Action dated January 3, 2002, claims 1-21 were rejected under 35 U.S.C. § 103(a) as allegedly obvious over Ogawa in view of Lee, Wan and Song. The Examiner alleges that it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to combine the method of purifying plasmid DNA of Ogawa with the methods of Lee, Wan and Song. Applicants respectfully disagree.

The Examiner is respectfully reminded that in order to find an invention *prima facie* obvious, the cited art must (1) teach or suggest each of the elements of the claimed invention, (2) provide suggestion or motivation to combine or modify the

references, and (3) provide a reasonable expectation that one could successfully arrive at the claimed invention. See M.P.E.P. § 2143 *et seq.* Applicants submit that the Examiner has failed to make his *prima facie* case because the references in combination do not teach each and every element of the claimed invention, and there is no suggestion or motivation to combine the references cited.

The cited art does not teach each and every element of the claimed invention.

The Examiner has failed to make his *prima facie* case of obviousness because the cited references, taken in combination do not teach each and every element of the claimed invention. **Specifically, the cited references fail to teach a neutralization step as it is defined in the specification and claimed in claim 1, step (f).**

The neutralization step is separate and distinct from the precipitation step. The purpose of the neutralization step is to help preserve the integrity of the plasmid DNA during the purification process by raising the pH of the solution containing plasmid DNA after the precipitation step to minimize acid catalyzed de-purination of the DNA and to condition the material for binding onto the anion exchange column (see specification, page 8, lines 3-6).

Applicants teach lysing the bacterial cells with an alkaline lysis solution and precipitating the denatured chromosomal DNA and cellular proteins by adding an acidic solution to the lysis mixture. A preferred alkaline lysis solution is 0.2 M NaOH/1% SDS and a preferred precipitation solution is 3 M potassium acetate pH 5.5. Applicants teach adding a neutralizing agent to either the acidic precipitation mixture prior to centrifugation or to the resulting clarified solution following centrifugation (see claim 1, step (f)).

The Examiner has not pointed out where Ogawa teaches neutralizing an acidic precipitation mixture. As stated by the Examiner, Ogawa teaches that the **alkaline lysate may be "neutralized"** by adding acetic acid or hydrochloric acid or by adding a

neutral buffer at a high concentration on page 2, column 2, lines 25-30. Ogawa further teaches in Example 1, page 3, column 4, lines 11-13 that a **preferred "neutralizing solution" to add to the alkaline lysate is 3 M sodium acetate pH 4.8.** This neutralizing solution is equivalent to the precipitation solution taught in the above referenced application. Once Ogawa's neutralizing solution has been added to the lysis mix, the sample is passed through a filter to remove the insoluble (precipitated) material prior to ultrafiltration (see column 2, lines 38-46 and column 4, lines 5-18). This filtration step is equivalent to the centrifugation step used by Applicants to remove the precipitated material from the sample after the precipitation solution is added to the lysis mixture. Once the sample is filtered to remove the insoluble material, Ogawa teaches ultrafiltering the sample containing plasmid DNA without any additional steps (see column 2, lines 47-56 and column 4, lines 14-18). Specifically, Ogawa does not teach adding a buffer to the sample before or after the insoluble material is removed and before further processing to increase the pH and prevent acid de-purination of the plasmid sample. The Examiner states in the Final Office Action in paragraph 13 on page 9 that the neutralizing step was inadvertently referred to as a precipitating step in an earlier office action. Applicants submit that the Examiner was correct in the earlier office action. The step which Ogawa calls a neutralization step is, in actuality, the same as the precipitation step taught by Wan and the above referenced application. Ogawa's neutralizing solution and neutralizing step is not equivalent to the neutralizing step taught in the instant application as discussed below.

The above referenced application claims that the acidic precipitation mixture of claim 1, step d is neutralized by the addition of a buffer solution preferably in the pH range of 7-8.5 (see specification page 8, lines 5-6) or by the addition of solid Tris base to raise the pH of the precipitation mixture to pH 8.5 (specification page 24, lines 2-3). As stated above, the purpose of this step is to prevent acid de-purination of the DNA in the sample. Ogawa does not teach or suggest this step. The failure of the teachings of Ogawa are not rectified by the teachings of Lee, Wan and Song. Applicants submit that the prior art cited by the Examiner do not teach a neutralization step as defined and

claimed by the above referenced application and therefore, the Examiner has failed to make his *prima facie* case of obviousness by failing to teach each and every element of the claimed invention.

There is no suggestion or motivation to combine the cited references.

Assuming *arguendo* the references cited by the Examiner did teach each and every element of the claimed invention, the Examiner also fails to make his *prima facie* case of obviousness because there is no suggestion or motivation to combine the references cited and there is teaching away. The Examiner states that the above referenced application is obvious over Ogawa in view of Lee, Wan and Song and that the motivation to combine these references may be found in the advantages each of the references teaches for purifying large scale plasmid DNA preparations. The Examiner relies on *In re Fine* 837 F.2d 1071 (Fed. Cir. 1988) and *In re Jones* 958 F.2d 347 (Fed. Cir. 1992) for stating that a teaching, suggestion or motivation to combine references may be found in the references themselves or in the knowledge generally available to one of skill in the art. Applicants submit that the Examiner has failed to point out where any of the references teach or suggest that they should be combined with any of the teachings of the other references. The Examiner has failed to point out the specific understanding or principle in the knowledge generally of one of skill that would motivate one of skill to make the specific combination of steps disclosed by Applicants.

The invention in the above referenced application is a method for purifying plasmid DNA for pharmaceutical use from bacteria on a large scale comprising a unique combination of steps. The Examiner has failed to point out where the cited art teaches assembling this unique combination of steps. The Examiner has failed to point out where the cited art teaches or suggests that static mixers, alkaline lysis, precipitation, neutralization and gel layers can be combined with centrifugation, ultrafiltration and ion exchange for large scale plasmid purification as taught by Lee. The Examiner has failed to point out where the cited art teaches or suggests that neutralization (as discussed above), centrifugation, static mixers, gel layers and ion exchange can be combined with

alkaline lysis, precipitation (as discussed above) and ultrafiltration for plasmid purification as taught by Ogawa. The Examiner has failed to point out where the cited art teaches or suggests that neutralization, centrifugation, ion exchange, gel layers and ultrafiltration can be combined with alkaline lysis and precipitation in static mixers for large scale plasmid purification as taught by Wan. The Examiner has failed to point out where the cited art teaches that static mixers, alkaline lysis, precipitation, neutralization, centrifugation and ion exchange can be combined with the formation of a gel layer during ultrafiltration as taught by Song.

The Examiner has cited three references, Ogawa, Lee and Wan, each of which teach a unique set of steps for purifying plasmid DNA. Each reference states that their protocol has advantages over other protocols. None of the references teach or suggest that their unique set of steps should be modified by additional purification steps.

The Examiner states that the motivation to pick and choose the steps chosen by the Applicants to purify plasmid DNA on a large scale from among the wide variety of steps available would have been obvious to those of skill because of the general knowledge of one of skill combined with the advantages each of the references teaches about the steps described in the reference. The Examiner has not pointed out any specific understanding or principle in the general knowledge of one of skill that would motivate one of skill to combine elements as done by Applicants to create the claimed invention. As such, the references are combined using improper hindsight.

In overturning a decision by the Board of Patent Appeals and Interferences, the court in *In re Fine* at 1074 agreed with the plaintiff that the PTO applied improper hindsight in combining the references without evidence to support the combination and in the face of contrary teachings in the prior art. The court stated:

> The PTO has the burden under section 103 to establish a *prima facie* case of obviousness. {cite omitted} It can satisfy this burden only by showing **some objective teaching in the prior art, or that knowledge generally available to one of skill in the art** would lead that individual to combine the relevant teachings of the references. {cites omitted} **This it has not done. The Board points to nothing in the cited references, either alone or in**

> **combination, suggesting or teaching Fine's invention.** . . . The Board reiterated the Examiner's bald assertion that "substitution of one type of detector for another in the system of Eads would have been within the skill of one in the art" but neither of them offered any support for this conclusion. [emphasis added] *Id.*

Here, the Examiner has not provided any objective teaching in any of the prior art references sited to make the combination of steps made in the above referenced application. The Examiner has not provided any evidence as to why one of skill would be motivated to combine the lysis procedure of Ogawa with the lysis, precipitation and static mixers of Wan, with the centrifugation, ultrafiltration and ion exchange of Lee, with the gel layer of Song other than stating that each reference teaches that the steps used in that particular reference have advantages.

The court in *In re Rouffet* 149 F.3d 1350, 1357-1358 (Fed. Cir. 1998) in overturning the rejection of Rouffet's invention based on cited art stated that a specific understanding or principle in the knowledge of the skilled artisan must be identified:

> **"[V]irtually all [inventions] are combinations of old elements."** . . . Therefore, an examiner may often find every element of a claimed invention in the prior art. If identification of each claimed element in the prior art were sufficient to negate patentability, very few patents would ever issue. . . . To prevent the use of hindsight based on the invention to defeat patentability of the invention, this court requires the examiner to show a motivation to combine the references that create the case of obviousness. . . . [T]he suggestion to combine requirement stands as a critical safeguard against hindsight analysis and rote application of the legal test for obviousness.
> Because the Board did not explain the **specific understanding or principle within the knowledge of a skilled artisan that would motivate one with no knowledge of Rouffet's invention to make the combination**, this court infers that the examiner selected these references with the assistance of hindsight. This court forbids the use of hindsight in the selection of references that comprise the case of obviousness.

Here, the Examiner has not explained the specific understanding or principle within the knowledge of one of skill in the art that would have motivated one of

skill to combine the cited references from among the vast array of references that describe plasmid purification techniques and to pick the specific steps from each reference to arrive at Applicants plasmid purification protocol.

It is well settled that the prior art cited by the Examiner must be considered in its entirety including sections that teach away from the invention. It is also well settled that it is improper to combine references when there is a clear teaching away from the combination.

Lee teaches away from the using alkaline lysis in large scale plasmid purification techniques. Lee teaches that plasmid purification techniques suitable for small or laboratory scale are not suitable for large scale isolation and purification of plasmid DNA and cites numerous limitations in laboratory scale plasmid purification techniques (Lee column 1, line 13 - column 2, line 32). Lee states that new technology must be developed to produce commercially viable large scale plasmid purification processes (Lee column 2, lines 33-46). It is improper to combine the teachings of Lee with the teachings of Ogawa because Ogawa is directed to alkaline lysis in small laboratory scale plasmid purification techniques.

The Examiner states that Ogawa is silent about the scale of the plasmid purification process disclosed. This is incorrect. Ogawa teaches in column 4, lines 2-13 that 1.5 mls. of a bacterial culture are spun down, the supernatant is removed and the cells are resuspended in 100 µl of a resuspension buffer, to which 200 µl of lysis buffer is added and 150 µl of precipitation solution is added for a total sample volume of 450 µl. These volumes are similar to the volumes typically used in a laboratory plasmid miniprep procedure. The above referenced application is directed to the purification of hundreds of milligrams of plasmid DNA from cells in 10s of liters of fermentation broth processed into 10s of liters of precipitation or neutralization mixture (see e.g. Example 5, pages 23-24). In view of the teaching of Lee that alkaline lysis in small laboratory scale plasmid purification procedures, such as those used by Ogawa, are not suitable for large commercial operations, it is improper to combine the teachings of Ogawa with the teachings of Lee.

The Examiner states that the motivation to combine the teachings of Song with the teachings of Ogawa, Wan and Lee is found in the theoretical background on the formation of gel layers in an ultrafiltration process provided by Song. The Examiner states that Song teaches that gel layer formation during ultrafiltration is a fundamental physical fact and any macromolecule must follow the laws of nature. The Examiner has failed to point out where Song teaches or suggests the use of a gel layer in a plasmid purification protocol and that the use of the gel layer is beneficial. The Examiner has also failed to point out the specific understanding or principle in the knowledge of one of skill that would motivate one of skill to combine the teachings of Song with the teachings of Ogawa Wan and Lee. The court has stated "the inherency of an advantage and its obviousness are entirely different questions. That which may be inherent is not necessarily known. Obviousness cannot be predicated on what is unknown." Application of Walter Spormann and Joachim Heinke 363 F.2d 444, 448 (CCPA 1966). The benefits of using a gel layer in a plasmid purification process as described in the above referenced application were unknown. Absent a teaching in Song that it is desirable, or beneficial to form a gel layer during a plasmid purification process it is immaterial that a gel layer may form inherently.

For all of the reasons stated above, the Examiner has failed to indicate the objective teaching in the prior art that would motivate one of skill to combine the references in the manner indicated by the Examiner to arrive at Applicants invention. The Examiner states that use of hindsight reasoning to combine references is proper if it only takes into account knowledge in the level of one or ordinary skill at the time the invention was made. As stated above, courts have held that the Examiner must point out the specific understanding or principle within the knowledge of the skilled artisan that would motivate one without knowledge of the applicants invention to make the same combination as the Applicants. The Examiner has failed to make his *prima facie* case of obviousness by failing to provide the suggestion or motivation to combine references in the manner suggested.

Obviousness type double patenting:

In the Final Office Action dated January 3, 2002, claims 18-21 were rejected under the judicially created doctrine of obviousness-type double patenting as allegedly unpatentable over claims 1-62 of U.S. Patent No: 6,011,148. Upon notification of allowable subject matter, Applicants will file a terminal disclaimer, disclaiming any term of any patent granted on the instant application which would extend beyond the expiration date of the full statutory term defined in 35 U.S.C. §§ 154-156 and § 173 of prior Patent No. 6,011,148 issued January 4, 2000, thereby obviating the obviousness-type double patenting rejection.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,

Peter Seperack
Reg. No. 47,932

TOWNSEND and TOWNSEND and CREW LLP
Two Embarcadero Center, 8th Floor
San Francisco, California 94111-3834
Tel: (415) 576-0200
Fax: (415) 576-0300
PKS
SF 1338832 v2

VERSION WITH MARKINGS TO SHOW CHANGES MADE

14. (Amended) The method of claim [1] 22, wherein steps (a), (b), (c), and (d) are carried out simultaneously.

15. (Amended) The method of claim [1] 22, wherein steps (a), (b), (c), (d) and (e) are carried out simultaneously.

16. (Amended) The method of claim [1] 22, wherein steps (a), (b), (c), (d) (e) and (f) are carried out simultaneously.

APPENDIX I

CURRENTLY PENDING CLAIMS

1. (Twice amended) A method for purifying plasmid DNA suitable for pharmaceutical use from bacterial cells on a large scale, the method comprising the following steps:

a) contacting bacterial cells which together comprise at least about 100 milligrams of the plasmid DNA with a lysis solution, thereby forming a lysis mixture;

b) flowing the lysis mixture through a first static mixer to obtain a lysed cell solution;

c) contacting the lysed cell solution with a precipitation solution;

d) flowing the lysed cell solution and the precipitation solution through a second static mixer, thereby forming a precipitation mixture;

e) centrifuging the precipitation mixture, thereby forming a pellet and a clarified solution comprising the plasmid DNA; and

f) neutralizing either the precipitation mixture prior to the centrifugation of step (e) or the clarified solution following the centrifugation of step (e);

g) contacting the clarified solution with a positively charged ion exchange chromatography resin, wherein the plasmid DNA is eluted from the ion exchange chromatography resin with a saline step or continuous gradient; thereby producing a solution of plasmid DNA of sufficient purity and quantity for pharmaceutical use, wherein the solution comprises at least about 100 mg of the plasmid DNA.

2. The method of claim 1, further comprising the step of RNase digestion.

3. The method of claim 1, wherein the lysis solution contains alkali.

4. The method of claim 1, wherein the precipitation solution contains potassium acetate.

5. The method of claim 1, wherein the neutralizing step precedes the step of centrifuging the precipitation mixture.

6. The method of claim 1, wherein the linear velocity of the lysis mixture through the first static mixer is between about 0.38 to 2.3 feet per second and the first static mixer has an outer diameter in the range of from about 3/16" inch to about 2 inches.

7. The method of claim 6, wherein the first static mixer has 24 elements.

8. The method of claim 6, wherein the first static mixer is a laminar flow static mixer.

9. The method of claim 1, wherein the linear velocity of the precipitation mixture through the second static mixer is between 0.38 to 2.3 feet per second and the second static mixer has an outer diameter in the range of from about 3/16 inch to about 2 inches.

10. The method of claim 9, wherein the second static mixer is a laminar flow static mixer.

11. The method of claim 9, wherein the second static mixer has 24 elements.

12. The method of claim 1, wherein steps (a) and (b) are carried out simultaneously.

13. The method of claim 1, wherein steps (c) and (d) are carried out simultaneously.

14. (Amended) The method of claim 22, wherein steps (a), (b), (c), and (d) are carried out simultaneously.

15. (Amended) The method of claim 22, wherein steps (a), (b), (c), (d) and (e) are carried out simultaneously.

16. (Amended) The method of claim 22, wherein steps (a), (b), (c), (d) (e) and (f) are carried out simultaneously.

17. The method of claim 16, wherein the method is automated.

18. (Amended) The method of claim 1, further comprising filtering the clarified solution through an ultrafiltration unit comprising a gel layer before contacting the clarified solution with the positively charged ion exchange resin.

19. The method of claim 18, wherein the ultrafiltration unit comprises a membrane having a molecular weight cutoff of from about 50K to about 500K daltons.

20. The method of claim 1, further comprising ultrafiltration of the plasmid DNA using tangential flow ultrafiltration with an open channel device, in the presence of a gel layer.

21. A method for purifying plasmid DNA suitable for pharmaceutical use from bacterial cells on a large scale, the method comprising the following steps:

a) contacting bacterial cells which together comprise at least about 100 milligrams of the plasmid DNA with a lysis solution, thereby forming a lysis mixture;

b) flowing the lysis mixture through a first static mixer to obtain a lysed cell solution;

c) contacting the lysed cell solution with a precipitation solution;

d) flowing the lysed cell solution and the precipitation solution through a second static mixer, thereby forming a precipitation mixture;

e) centrifuging the precipitation mixture, thereby forming a pellet and a clarified solution comprising the plasmid DNA; and

f) neutralizing either the precipitation mixture prior to the centrifugation of step (e) or the clarified solution following the centrifugation of step (e);

g) contacting the clarified solution with a positively charged ion exchange chromatography resin, wherein the plasmid DNA is eluted from the ion exchange chromatography resin with a saline step or continuous gradient; and

h) filtering the clarified solution of step (f) through an ultrafiltration unit comprising a gel layer either before or after contacting the clarified solution with the positively charged ion exchange resin of step (g), thereby producing a solution of plasmid DNA of sufficient purity and quantity for pharmaceutical use, wherein the solution comprises at least about 100 mg of the plasmid DNA.

22. (New) The method of claim 1, wherein the steps are performed in a continuous process on a single large volume sample.